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GGAAGAAAACCTGGTAGTACTAGTTCACCTAAGGCCTTATCACCTCCTCCTTC
TGTGGATTCAAATTACCCAACGAGAGATAGAGCATCTTTCAACAGAATGGTC
ATGCATAGTGCTGCTTCTCCAACACAGGCACCAATCCTTAATCCCTCTATGGT
TACAAATGAAGGACTTGGTCTTACAACTACAGCTTCTGGAACAGACATCTCTT
CTAATAGTCTAAAAGATTGTCTTCCTAAATCAGCACAACTTTTGAAATCTGTT
TTTGTGAAAAATGTTGGTTGGGCTACACAGTTAACTAGTGGAGCTGTGTGGGT
TCAGTTTAATGATGGGTCCCAGTTGGTTGTGCAGGCAGGAGTGTCTTCTATCA
GTTATACCTCACCAAATGGTCAAACAACTAGGTATGGAGAAAATGAAAAATT
ACCAGACTACATCAAACAGAAATTACAGTGTCTGTCTTCCATCCTTTTGATGT
TTTCTAATCCGACTCCTAATTTTCATTGA

>SAK amino acid seq. (SEQ ID NO:2)

MATCIGEKIEDFKVGNLLGKGSFAGVYRAESIHTGLEVAIKMIDKKAMYKAGMV
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LYLHSHGILHRDLTLS
NLLLTRNMNIKIADFGLATQLKMPHEKHYTLCGTPNYISPEIATRSAHGLESDVW
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GWATQLTSGAVWVQ
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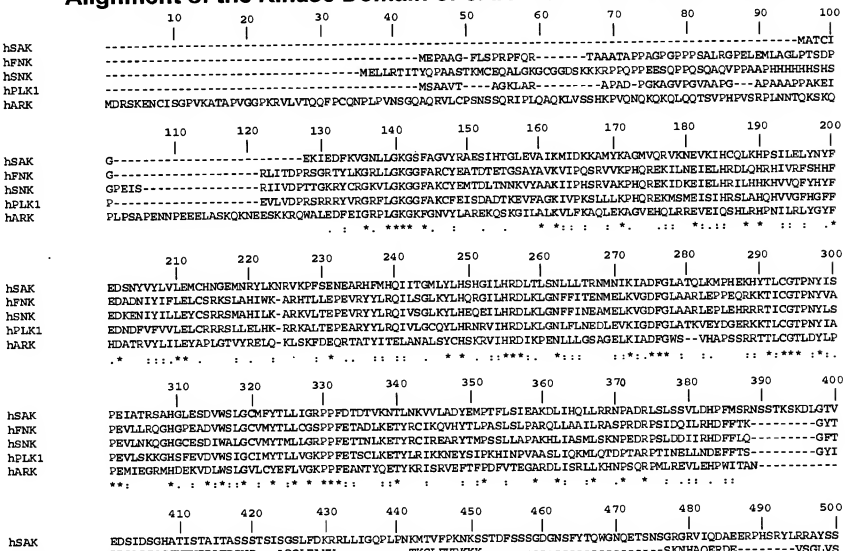
PNFH

Alignment of the Kinase Domain of SAK with Other Mitotic Kinases



Two hSAK Mutants Generated for the Dominant negative Studies: D154A and K41M

FIG. 2.

Summary of Target Validation Studies: SAK

Dominant negative studies

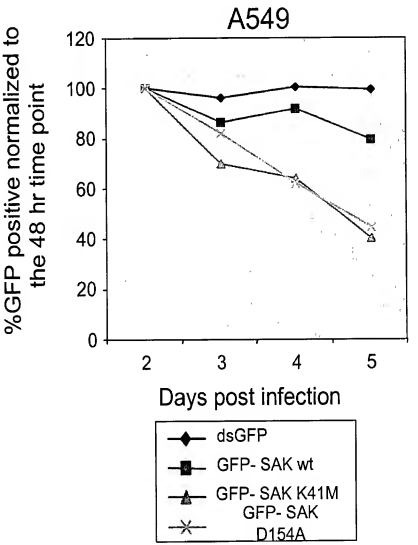
Antiproliferative Activity	Tumor A549	Hela	PC-3	MCF7	H1299	Normal HMEC	PrEC
Wt							
GFP fusion	+	+	++	+/-	+/-	+/-	+/-
IRES GFP	+	+		+/-	nd	+/-	nd
K41M							
GFP fusion	++	++	++	+	+/-	+/-	+/-
IRES GFP	++	++	++	+	nd	+/-	nd
D154A							
GFP fusion	++	nd	++	+	+/-	+/-	+/-
IRES GFP	++	nd	++	+	nd	+/-	nd

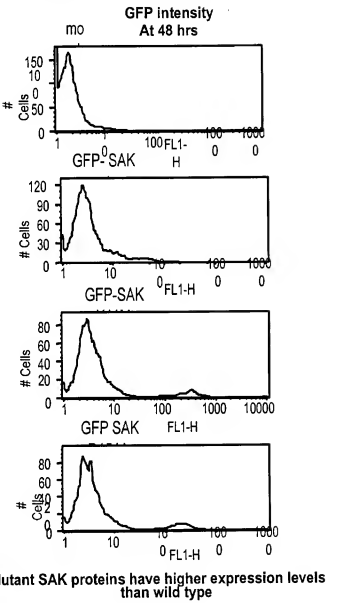
Antisense:	Hela	A549	H1299
	+	+/-	+/-

(+ indicates antiproliferative effect in either the GFP positivity study, cell tracker or antisense studies)

FIG. 3

Overexpression of SAK Mutants Have a More Pronounced Antiproliferative Effect than Wild Type in A549 Cells





Mutant SAK proteins have higher expression levels than wild type

FIG . 4

SAK Mutants Have a More Pronounced Antiproliferative Effect Relative to Wild Type in A549 Cells

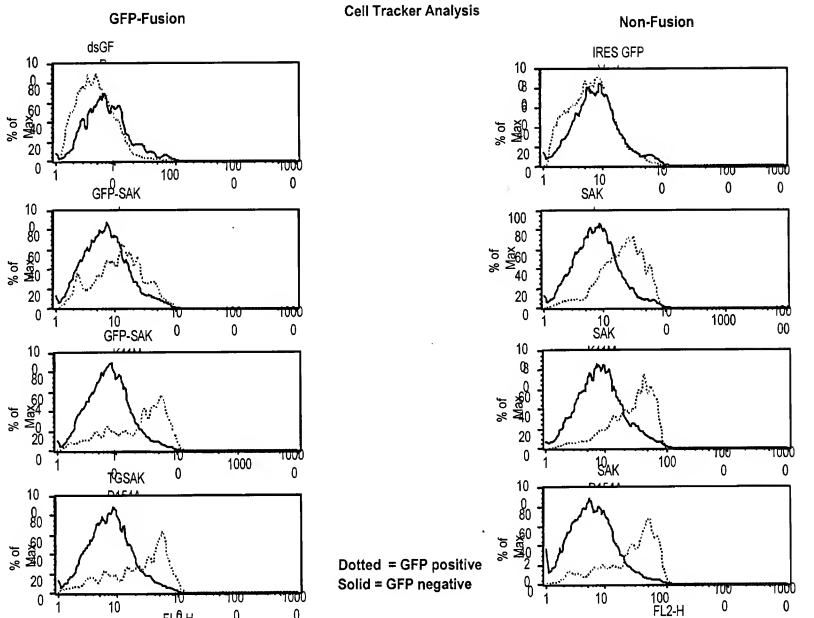


FIG. 5

SAK Mutants Have a More Significant Antiproliferative Effect Than Wild Type in MCF7 Cells

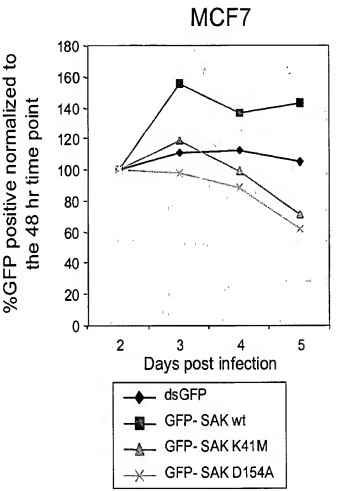


FIG. 6

SAK Wild Type and Mutants Have Similar Antiproliferative Effects in PC-3 Cells

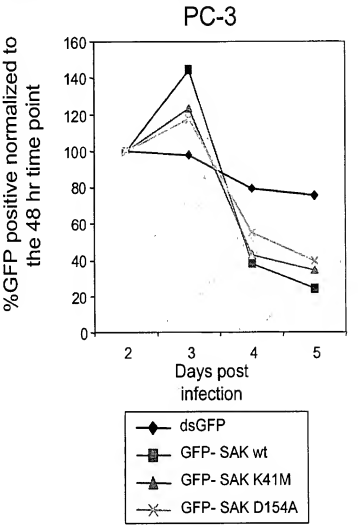


FIG. 7

SAK K41M Mutant has a Weak Antiproliferative Effect in H1299 Cells

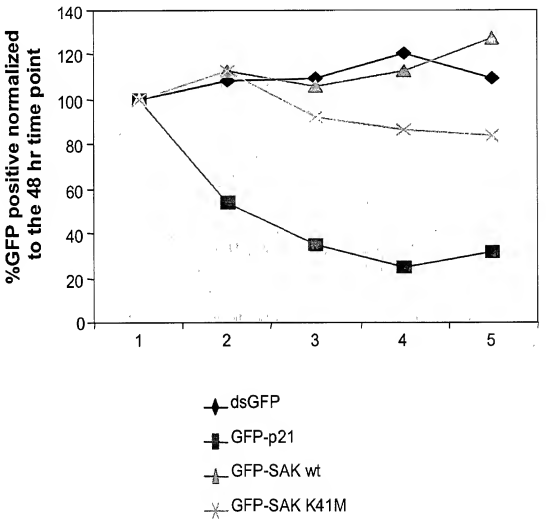


FIG. 8

SAK Wild Type and Mutants Have No Antiproliferative Effects in Normal Cells in GFP Positivity Studies

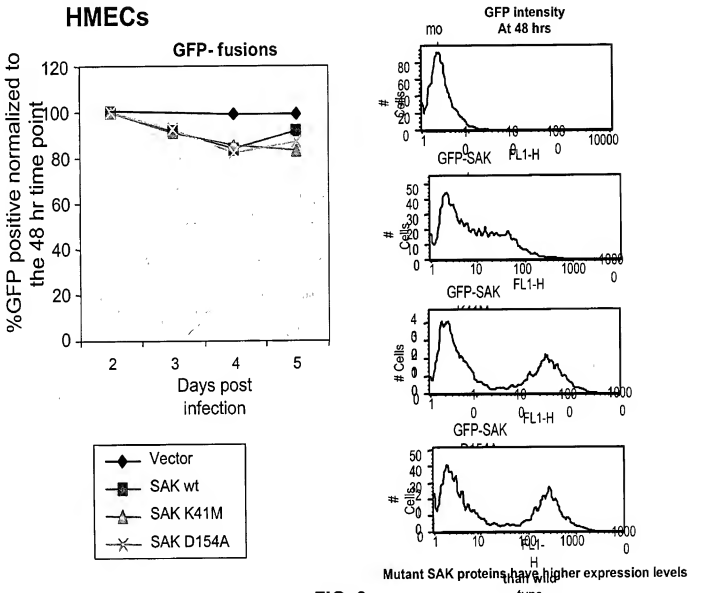
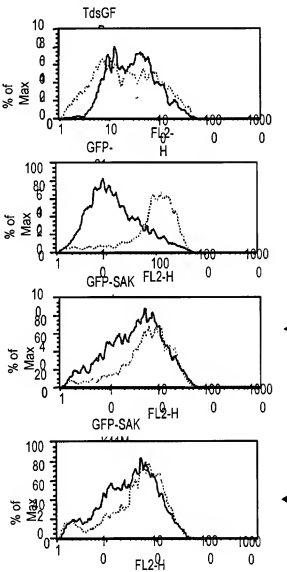


FIG. 9

SAK Wild Type and Mutant Proteins Do Not Have Significant Antiproliferative Activity in Normal Cells

HMECs



Cell Tracker Assay

5 days post infection

Dotted = GFP positive
Solid = GFP negative

No significant antiproliferative effect

No significant antiproliferative effect

FIG. 10

SAK K41M Mutant Does Not Have Strong Antiproliferative Effects in Normal Cells

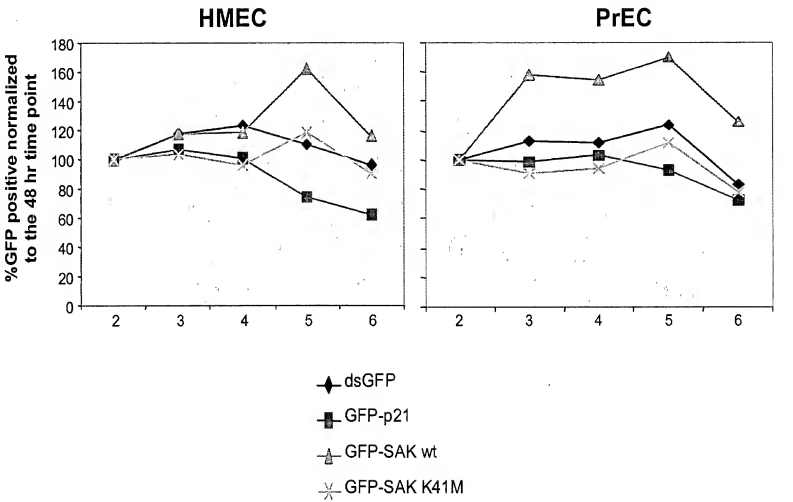
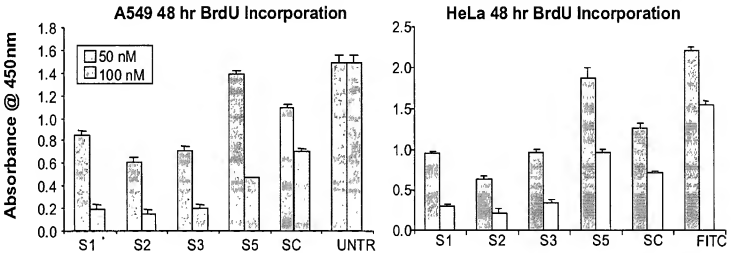


FIG. 11

Reduction of SAK With Antisense Oligo Transfections is Antiproliferative in HeLa and A549 Cells



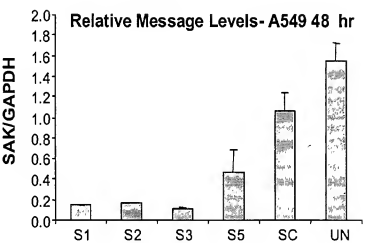


FIG. 12

Reduction of SAK With Antisense OligoTransfections is Weakly Antiproliferative in Huvec Cells

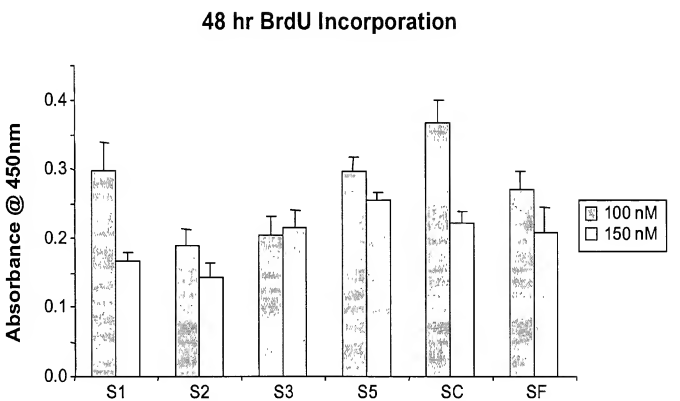
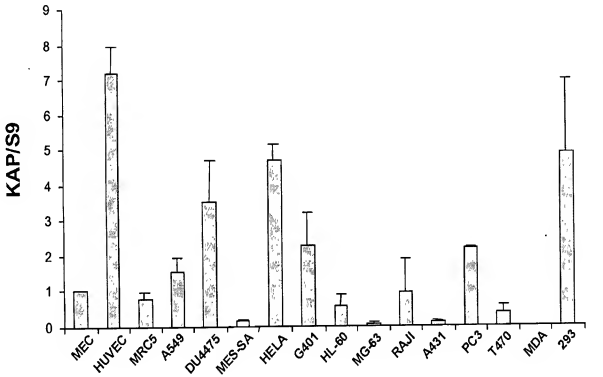


FIG. 13

SAKmRNA is Overexpressed in Some Tumor Cell Lines



MEC	Mamamary Epithelium
HUVEC	Umbilical Cord Endothilium
MRC5	Embryonic lung fibroblast
A549	Small-cell lung carcinoma
DU4475	Breast carcinoma
MES-SA	Uterine sarcoma
HELA	Cervical carcinoma
G401	Wilm's tumor
HL-60	Myeloid leukemia
MG-63	Osteosarcoma
RAJI	Burkitt's lymphoma
A431	Epidermal carcinoma
PC3	Prostate carcinoma
T470	Breast carcinoma
MDA-MB-361	Breast Adenocarcinoma
293	Embryonic kidney

FIG. 14

SAK Summary

Identification

Proteomics- Chk2 interacting protein

Functional Studies

Dominant Negative Studies

- Mutant SAK has a much stronger antiproliferative phenotype than the wild type SAK in tumor cells while neither wild type or mutant SAK is antiproliferative in normal cells.
- The higher expression level of the mutant SAK relative to wild type makes it difficult to validate SAK only by the dominant negative strategy

Antisense Studies

- Preliminary studies suggests that inhibition of SAK mRNA with antisense oligos is antiproliferative in A549 and Hela cells

Literature

- Strong supporting literature shows antisense reduction of mouse SAK is antiproliferative and that the mouse SAK knockout results in increased cell cycle arrest and apoptosis

FIG. 15

Model for Antiproliferative Activity Associated with SAK Inhibition

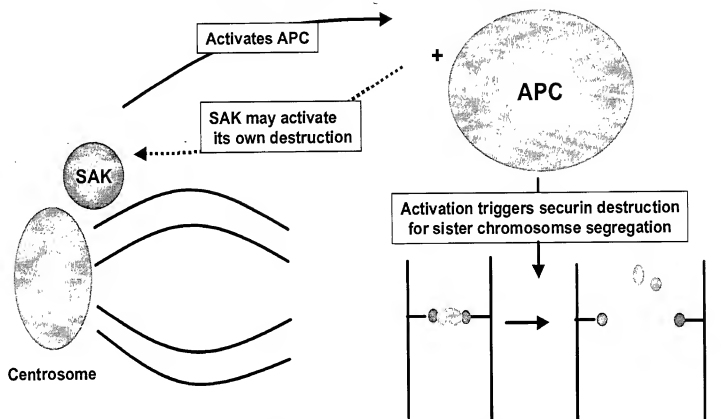


FIG. 16

Biochemical assay for Sak kinaseactivity

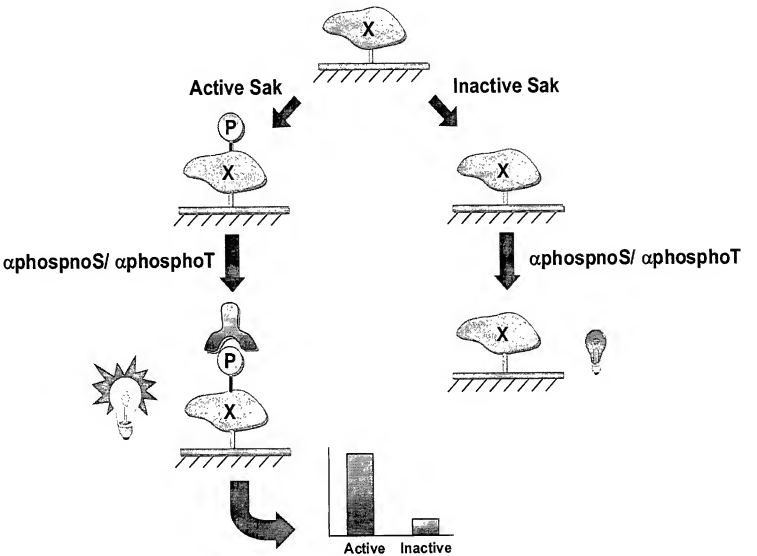


FIG. 17

Protocol for Sak Autophosphorylation Assay

Bind Sak from *E. coli* lysates to Ni-NTA agarose O/N at 4°C

Wash Ni-NTA with lysis buffer (20 mM Hepes,pH 7.2, 0.5 M NaCl, 0.5% Tween-20, 25 mM β-glycerol phosphate, 1 mM NaF, 1 mM Na_3VO_4, 1 mM NaPyP, 10% glycerol

Wash Ni-NTA with kinase buffer (20 mM MOPS, pH 7.2, 25 mM β-glycerol phosphate, 5 mM EGTA, 1 mM Na_3VO_4)

Resuspend resin-bound Sak in 10 µL kinase buffer
Add 10 µL of labeling mix (20 mM $MgCl_2$, 2 mM $MnCl_2$, 0.2 mM ATP, 0.5 µCi/µL γ-^{32}P ATP in kinase buffer
Incubate at 30°C, 15 min.

FIG. 18

Autophosphorylation Activity of Sak Produced in *E. coli*

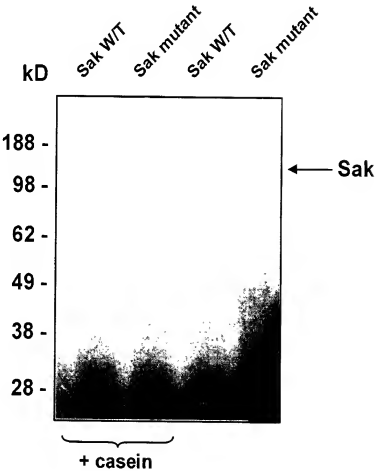


FIG. 19